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(54) SILVER ALLANTOINATE-CONTAINING COMPOSITIONS

(71) We, WASHINGTON UNIVERSITY, a corporation organized under the laws of the State of Missouri, United States of America, of Skinker and Lindell Boulevards, St. Louis, Missouri, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

The present invention is concerned with a composition containing silver allantoinate which has been found to be particularly useful for topical application in the treatment of burn wounds to promote wound healing and antiseptic prophylaxis.

According to the present invention, there is provided a pharmaceutical composition comprising silver allantoinate of the formula $C_4H_6O_3N_4Ag$ and having a water solubility of about 0.02% (weight per volume) at 25° C and a pharmaceutical carrier.

Preferred compositions according to the invention are those in which the silver allantoinate is present in an amount of from 0.1 to 3.0 grams per 100 grams of carrier.

The present invention also comprises a method of preparing silver allontoinate, which comprises adding an aqueous solution of silver nitrate to an aqueous solution of allantoin, the weight of silver nitrate used being substantially equal to the weight of the allantoin, then adding ammonia to form a precipitate, and separating the precipitate consisting of silver allantoinate of the formula $C_4H_6O_3N_4Ag$ and having a water solubility of about 0.02% (weight per volume) at 25° C.

Hereinafter, all solubilities are expressed on a weight per volume basis.

The use of silver allantoinate for medical purposes in respect of its general germicidal and healing qualities has been described in U.S. Patent No. 2,336,131 (Schaffer). The process described in the Schaffer patent for the preparation of silver allantoinate gives a low yield of about 25% and the product has a relatively high water solubility of 0.4% at 25° C. In contrast, the process according to the invention for the preparation of silver allantoinate gives yields of about 95% and the product has a water solubility of only about 0.02% at 25° C; this product is obtained in the form of a stable white powder which has a good shelf life.

The composition according to the invention has good bacteriostatic and healing qualities substantially unaccompanied by skin discolouration, commonly known as localized argyria. The composition can be used on burn wounds without serious loss of electrolytes from the body and without the very close control required in the silver nitrate treatment for burns. The cost of the composition is relatively low and incorporation of the silver allantoinate into the carrier is easily effected, any of the common ointment, cream, lotion or aerosol spray carriers being suitable.

As a silver ion ($Ag+$) approaches another ion or molecule X, the more loosely held electrons can be attracted toward X (polarization) and perhaps even shared to some extent with X (formation of partially covalent bond). As a result of this added attraction, the silver ion forms many complex ions and many precipitates.

Silver is reluctant to combine with oxygen. The oxide is made not by direct union of the elements, but by precipitation. The solubility of silver oxide is abnormally large in strongly basic solutions. This increase in solubility has been attributed to the reaction of Ag_2O as an acid to give AgO^- or $Ag(OH)_2^-$), a behavior which is called amphoteric. Together with allantoin, another amphoteric substance (which forms alkali salts behaving as a weak acid and forms salts with acids behaving as a weak

base), it may therefore form both salts and complexes.

The antibacterial action of inorganic silver salts and of silver-protein is due to free silver ions. Microorganisms take up silver ions readily and the proteins of bacterial protoplasm are precipitated. The formed silver proteinate continues to liberate silver ions within the bacterial cell, and these silver ions are capable of reacting with critical macromolecules of the cell.

Silver is known to form complexes with polynucleotides. The silver ion combines in equimolar ratio with guanosine 3′ - monophosphate inosine, inosine 5′ - monophosphate and the theophylline. It forms also a complex with riboflavin at pH 7.

Silver allantoinate is much less soluble in water than inorganic silver salts such as the nitrate, or organic salts such as the lactate, i.e. in the absence of strong base or acid ionization is limited. Despite this, when corresponding amounts of silver in their respective anionic combination were tested *in vitro* as to their bacteriostatic effectiveness i.e. their ability to inhibit bacterial growth of common pathogenic organisms, the silver allantoinate was at least twice as effective as its closest competitors, silver chloride and silver citrate, two other silver compounds of very limited water solubility.

The effectiveness of silver allantoinate appears to depend on the oligodynamic action of a limited number of silver ions, set free only at a slow release rate from the silver allantoinate due to its low solubility. This slow release rate corresponds to the utilization of silver ions through chemical binding and adsorption to bacteria and body protein. The maintenance of an equilibrium, i.e. a constant silver ion concentration, is assured by the presence of sufficient complex from which, upon demand, active silver ions can be obtained. By this action loss of silver is minimized while its high rate of effectiveness is established.

Various forms of silver, including the pure metal, certain of its derivatives and colloidal suspensions thereof have been known as antiseptic or germicidal agents for several centuries.

The bactericidal power of metallic silver is known for its oligodynamic activity, which term is used to describe the lethal properties of any metal which exhibits anti-microbial properties even in minute concentrations. Metallic silver, i.e. silver in non-ionized form, apart from possessing antiseptic properties, is also non-toxic. Accordingly, it has found practical application as an antiseptic. It has been used in various forms, most often as the colloidal suspension. In addition, silver has been used for bactericidal purposes in the form of various salts, for example, silver nitrate, silver citrate, silver lactate, silver picrate, silver chloride and silver proteinates.

A large number of compounds and complexes of silver are known and have found use as analytical, catalytical and sometimes antimicrobial agents. In the case of the latter function many silver derivatives are not suitable for use, topically or otherwise, in humans because of either extremely adverse physical properties or the toxicity of the associated anion. For instance, silver chloride has a very slight water-solubility but on exposure to light, it immediately turns black. The sodium-argento-thiosulfates on the other hand, are very soluble, most effective as antibacterial solutions, but they have proven to be rather toxic even when applied topically.

Although silver nitrate is used in concentrations that vary from 0.5% to 10% or more, it has corrosive and irritating properties. The nitrate ion has been found to convert to the nitrite ion, especially in the presence of bacteria, and as such, it is toxic and may lead to methemoglobinemia, which may be fatal, especially in young patients.

In addition, the high solubility of silver nitrate may render its use dangerous because of the ability of silver ions to deplete the body of electrolytes, i.e. negatively charged ions such as the chloride and with it carry other, life-important elements such as sodium, potassium, magnesium and calcium.

Accordingly, close supervision of burn patients treated with silver nitrate is necessary. Use, therefore, is limited to larger, well equipped medical centres, and application to casualties in the field is impossible. Repeated microchemical analyses are needed for effective therapeutic control of patients treated with solutions of silver nitrate. The same objections apply to the use of certain other, water-soluble silver salts, such as the lactate or acetate, despite the more physiological character of these anions.

The bactericidal properties of silver in its many forms have been theoretically attributed to the micro-solubility of the metal in water with the consequent production of active metal ions and the adsorption thereof by the bacteria and resultant protein coagulation. It is generally known that metallic silver is less active, for example, than its salts and that the chemically pure metal is completely inactive.

Extensive research into the mode of action of silver as a germicidal agent has resulted in a number of theoretical considerations which are described below. The bacteriostatic and bactericidal effects of silver are closely related to the chemical and physical behaviour and not to the actual number of silver ions. Free silver ions are effective bacteriologically as long as there is a sufficiently large reserve or depot of non-ionized silver available to maintain a constant silver ion concentration. In this respect, the low solubility of the silver allan-

toinate composition prepared by the process herein described is advantageous. The concentration limit at which a silver compound can kill a certain number of microorganisms depends on its silver content and is proportional to it, and also on the number and power of adsorption of different microorganisms. The rate with which silver ions that are released from the "depot" that act upon and are "lost" to the bacteria, are replaced, determines the rate at which bacteria are destroyed. Silver in the various silver compositions is dissolved by certain products of bacterial metabolism more readily than by others. Lactic acid is one of the products which yields silver lactate while ammonia is another and yields other silver complexes.

The antiseptic effect of the silver in the composition can be seen to take place in two distinct steps. In the first, the silver ions react with the life important component of the microorganism, while in the second silver ions react with components in the system, such as sodium chloride, protein and the like. The first step takes place with all silver compounds or complexes in an identical manner and in the treatment no influence or choice can be exercised. In the second step, it is known that a reaction occurs at different rates with different compounds and on this rate depends the bacteriological effectiveness.

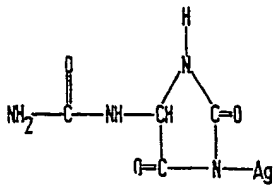
In the presence of different proteins, the distribution of silver depends on the quantity and the respectively binding capacity of the proteins. Thus, there is a selective effectiveness of silver, depending on the presence or absence of certain specific organisms, i.e. bacteria, fungi, or viruses. It has been proven that silver binds with relative ease to albumin but not to globulin. The silver protein binding is reversible in the presence of compounds for which silver has an even greater affinity, for example, compounds containing a —SH(mercapto) grouping.

The equilibrium between ionized and complexed silver greatly influences the anti-microbial effectiveness of silver-containing compositions. In organo-metallic combinations in which the metal atom has entered the more or less stable structure of organic compounds, the specific effect of the metal disappears and the organo-metallic molecule, as a whole, determines the effect. Silver-salvarsan and silver-sulfadiazine art typical of such compounds. On the other hand, compounds such as the argentamines, despite their organic components, belong to the class of complex salts. Silver allantoinate is another such example.

Considering silver allantoinate as a chelate of a nitrogenous base (Allantoin=5-Ureidohydantoin) and ionic silver, the concept that it acts by distortion of the DNA structure and by alteration of the microbial DNA function, thus inhibiting cell proliferation, must be considered most likely.

Silver allantoinate may exist in (at least) two different structural forms. The silver allantoinate employed in this invention is believed to have the structural formula 1 below, where the silver may be in the 1 or 3 position and the solubility in water is 0.02% at 25° C:

1)



M.W. 265
Ag+=40.5%±0.2%

3 Silver Allantoin

2)

$\left[Ag_2O:(C_4H_6O_3N_4)_2\right]$ Silver oxide: Allantoin Complex

M.W. 548
Ag+=39%

The silver allantoinate compositions of the present invention are advantageous when employed as a silver burn cream or ointment. The advantages include promotion of burn healing and lack of retardation coupled with a low sensitization index and a low index of irritation. The silver allantoinate is non-dehydrating, has good pharmaceutical properties and lends itself to incorporation in carriers, such as ointments, creams, lotions, and especially those that are non-greasy. The dry silver allantoinate has a long shelf life and is compatible with other chemical ingredients commonly used in pharmaceutical carriers. Furthermore, despite the low solubility of the silver allantoinate, the compositions show an efficient release of the silver allantoinate as a therapeutic agent at the site of application to the burn wound and are effective over a long period of time. Because of its physical properties and non-greasiness, the silver allantoinate is easily removed with water. It further is desirable in chemical formulating and ease of compounding since it can be used as a single ingredient along with the carrier. The compositions may be applied to the burn area with minimum resultant sensation of pain during

the treatment. The ease of procurement of the ingredients used in the compositions and their relatively low cost represent further advantages.

In order that the invention may be more fully understood, the following examples are given by way of illustration only.

EXAMPLE I.

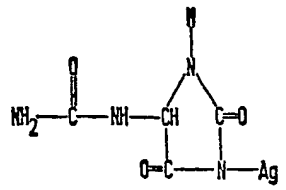
Silver allantoinate with a water solubility of 0.02% at 25° C was prepared as follows.

100 gm. allantoin were dissolved in 2 litres of boiling distilled water and to this solution was added 100 gm. silver nitrate in the form of 200 ml. of a 50% aqueous solution. This addition was made rapidly with constant stirring, and the resulting solution was clear and colourless.

Ammonia in the form of ammonium hydroxide (28—29%) was added dropwise, with stirring, until further addition did not produce a precipitate. Approximately 50 ml of ammonia was utilized. The mixture was permitted to cool and was filtered through a Buechner filter under suction. The precipitate was packed, washed with cold distilled water containing 1% hydrogen peroxide, spread on filter paper and dried in air over night. Finally, the precipitate was powdered and, preferably, micronized and stored in a well closed and taped bottle.

The yield from the process was approximately 150 gm. of silver allantoinate and represented 95% of the theoretical yield. The silver content was 40.5±0.2 percent by weight.

It is believed that the silver allantoinate is in the form of an organo-metallic compound of the empirical formula $C_4H_5O_3N_4Ag$ having the structural formula as set forth below:



SILVER ALLANTOINATE

As prepared in the process of Example I, the silver allantoinate has a solubility in water of 0.02 gm/100 ml at 25° C. It is insoluble in ethanol, acetone and glycerol but soluble in alkalis, such as ammonia, 2 - amino - 2-methyl - 1, 3 - propanediol, 2 - amino - 2-ethyl - 1, 3 - propanediol, tris - hydroxy-aminomethane, triethanolamine, and slightly soluble in organic acids, such as lactic acid, citric acid and acetic acid, apparently through further complex formation. The hydrogen peroxide prevents any reduction of the ionic silver and resultant discoloration and prolongs the shelf life of the product and of the finished cream when the product is incorporated in such a carrier.

The silver allantoinate thus prepared was evaluated for inhibition of growth of bacteria by a modification of the method of Reddish. In this method, 200 mg. samples were weighed, representing approximately 80 mg of silver, partially in ionic form. All tests were made in quadruplicate and mean values are shown as follows:

	Pseud. aerug.	Staph. aureus
Silver Allantoinate	12 mm.	12 mm.

Against both organisms tested, the zone of inibition of growth was 12 mm.

The silver allantoinate employed in the compositions of this invention, and having a water solubility of 0.02% at 25° C, was compared with the silver allantoinate having a water solubility of about 0.4% at 25° C and prepared in 25% yield, described in U.S. Patent No. 2,336,131 (Schaffer). The silver allantoinate made according to the Schaffer patent showed a zone of inhibition as follows:

Schaffer	Pseud. aerug.	Staph. aureus
Silver Allantoinate	13 mm	12 mm

Thus is appears that the effectiveness of the silver allantoinate employed in the present invention, as compared with the Schaffer silver allantoinate, against the identical test organisms is essentially the same, despite the greater solubility of the Schaffer composition. The Schaffer composition is essentially that set forth below:

$C_4H_5N_4O_3Ag\ OH\ (38.2Ag)$

The silver allantoinate prepared by Example I is advantageously incorporated into an antiseptic burn cream having a pH of 7.5. This burn cream, which is applied topically to the burn area, may be prepared as described in Example II.

EXAMPLE II

Stearyl Alcohol	200 Gm.	
Cetyl Alcohol	100 Gm.	Mixture I
Myristyl Alcohol	100 Gm.	
Petrolatum USP	320 Gm.	
Mineral Oil, light, incl. dye*	50 ml.	melt at 70°C
Polyethylene Glycol 600	180 ml.	
Propylene Glycol	40 ml.	Mixture II
Squalane	20 ml.	
Silver Allantoin-Complex** (approx.)	2000 ml.	45°C.
Allantoin	50 Gm.	
Sodium Lauryl Sulfate	10 Gm.	Mixture III
Distilled Water	g.s. 2500 ml.	

*) Scarlet Red, dissolved 500 mg/500 ml light mineral oil (stock). Use 15 ml, q.s. to 50 ml with light mineral oil.

**) 2-amino-2-methyl-1, 3-Propanediol	280 Gm.
Silver Allantoinate	280 Gm.
Distilled Water	1440 ml.
	2000

The mixture was allowed to stand with occasional shaking, until a clear solution was obtained, which was filtered through a *Watman* No. 2 filter paper (Watman is a trade mark).

To 800 ml of this solution was added 300 ml oleic acid and 800 ml distilled water and the mixture was neutralized by blending.

Mixtures I, II and III and the silver allantoin complex were prepared from the ingredients listed above.

Mixture I was melted at 70° C and mixture II was then added to it. The silver allantoin complex at 45° C was washed into the mixture of I and II with water (100—200 ml). Mixture III was then slowly added with stirring. Stirring was continued until the resultant mixture had cooled, when it was homogenised after addition of 50 ml 3% H_2O_2.

Active Ingredients:

Silver: approx 0.8%

Allantoin: approx. 2.1% of which 1.2% is combined allantoin.

The free allantoin in Mixture III is employed to provide an excess of allantoin to stabilize the silver allantoinate and also to provide free allantoin to promote healing of the infected area.

The bactericidal effectiveness of silver increases with increasing pH, i.e. silver ions are

less effective at lower pH suggesting a competition between metal cations and hydrogen ions for anion sites on the bacteria.

The silver allantoinate may be incorporated into compatible ointment or cream bases low in fat where burn treatment is contemplated, such as US Pharmacopea hydrophilic ointment base. Such incorporation is preferably in micronized form i.e. small particle size such as 100 to 200 mesh as 100 microns in size or smaller.

Another example of incorporation of silver allantoinate in a burn cream is described in Example III.

EXAMPLE III

Mixture 1

Cetyl Alcohol	400 Gm	melt at 75°C
Glycerol	200 ml	
Propylene Glycol	40 ml	
Polyethylene Glycol 600	80 ml	
Squalane	10 ml	
Mineral Oil incl. dye	20 ml	

Mixture 2

Distilled water	2300 ml	
Allantoin	20 Gm	
Sodium Lauryl Sulfate	20 Gm.	heat to 50°C.

Mixture 3

Triethanolamine	30 ml.
Silver Allantoinate	30 Gm.
Dist. water	30 ml.

Mixture 2 was stirred into Mixture 1. Mixture 3 was then washed into mixtures 1 and 2 the resultant mixture stirred until cold.

Hydrogen peroxide (500 ml, 1%) was added, the mixture was again stirred and then placed in jars.

Silver: 0.33%
Allantoin: 0.6%
pH: 7.8

Allantoin has a long history of therapeutic value which is closely connected with its natural predecessor "comfrey root". Chemically, allantoin is a uric acid derivative (diureide of glyoxyllic acid; also known as 5-ureidohydantoin or glyoxyldiureide) and is commercially available at low cost.

The silver allantoinate may be added in micronized or powder form to a number of water soluble carrier bases, such as ointments, lotions, creams and aerosol sprays, where the components are compatible with silver, preferably in concentrations from 0.1 to 3 gms per 100 gms of the ointment or other base. In these concentrations, in contrast to silver nitrate, there is no danger of regenerating apithelial cells. The effectiveness varies from bacteriostatic to bactericidal as the silver concentration increases. The low water solubility of the silver allantoinate permits the use of a concentration higher than actually necessary, which has the advantage of being able not only to control existing topical bacterial or fungal infections, but also to prevent their occurrence.

An advantage of treating burn wounds with

the burn ointment or cream of the invention is that the supplemental oral salt intake required to overcome electrolyte deficiency is much less than when 0.5% aqueous solutions of silver nitrate are used. Also, methemoglobinemia does not occur, and the topical treatment with the burn cream is at least as effective as the employment of 0.5% silver nitrate solution in controlling burn wound infection. Additionally, staining of floors, walls and bed clothing and the skin is substantially reduced when the silver allantoinate cream is employed rather than silver nitrate solution.

The treatment of the burn wound is instituted by removing all loose skin from the burn wound surface, which requires excision of all blisters and loose skin and wiping off of non-blistered skin which overlies the burns. Swab wipings for bacterial culturing are obtained from the various parts of the wound at this time and put into appropriate culture media. All grease and ointments that may have been applied to the wound elsewhere are removed. Occasionally immersion in a bath of warm Locke's solution or other appropriate solution is necessary to accomplish the removal of all such ointments.

With the burn wound thus prepared, the silver allantoinate burn cream is applied over the entire burn area. Following the liberal application of the cream, sterile gauze dressings are applied over the ointment. A stockinette may then be wrapped snugly over the dressings to hold them in place.

Dressings are changed periodically, when the burn wound is inspected carefully and all loose eschar or excess ointment is removed. The burn cream ointment is water miscible so most of it may come off with the dressings. The burn wound is then again covered with ointment and dressed as previously described. Bacterial cultures of the burn wounds are secured two or three times weekly and quantitative culture counts determined. The dressings and debridement are continued until the wounds heal and are ready to receive cutaneous autographs. Due to the "depot" effect of the silver allantoinate burn cream, the frequency of dressing changes may be reduced from the usual twelve hours to twenty-four hours or more. This represents a significant advantage to the burn patient and the treating personnel.

WHAT WE CLAIM IS:—

1. A pharmaceutical composition comprising silver allantoinate of the formula $C_4H_6O_3N_4Ag$ and having a water solubility of about 0.02% (weight per volume) at 25° C in a pharmaceutical carrier.

2. A composition according to claim 1 in which the silver allantoinate is present in the amount of 0.1 to 3.0 grams per 100 grams of carrier.

3. A composition according to claim 1 or 2 in which the pharmaceutical carrier is water soluble.

4. A composition according to claim 1 or 2 in which the pharmaceutical carrier is hydrophilic.

5. A composition according to any of claims 1 to 4 in which silver allantoinate is present in the pharmaceutical carrier in the form of a powder.

6. A composition according to any of claims 1 to 5 which additionally includes allantoin.

7. A method of preparing silver allantoinate, which comprises adding an aqueous solution of silver nitrate to an aqueous solution of allantoin, the weight of silver nitrate used being substantially equal to the weight of the allantoin then adding ammonia to form a precipitate, and separating the precipitate consisting of silver allantoinate of the formula

$$C_4H_6O_3N_4Ag$$

and having a water solubility of about 0.02% (weight per volume) at 25° C.

8. A method of preparing silver allantoinate substantially as herein described in Example I.

9. Silver allantoinate when prepared by the process according to claim 7 or 8.

10. A pharmaceutical composition containing silver allantoinate substantially as herein described in Example II or III.

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